Resuscitation 82 (2011) 1453–1459

Contents lists available at ScienceDirect

Resuscitation

journal homepage: www.elsevier.com/locate/resuscitation

Experimental paper

Severe controlled hemorrhage resuscitation with small volume poloxamer 188 in sedated miniature swine☆,☆☆

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ARTICLE INFO

Article history:
Received 9 May 2011
Accepted 8 June 2011

Keywords:
60% hemorrhage
Thromboelastogram (TEG)
Survival
Surfactant
Metabolic acidosis
Hypotension
Coagulopathy

ABSTRACT

Background: The surfactant poloxamer 188 (P188) has been shown to improve survival following hemorrhage. This study used P188 as a small volume resuscitation product in a sedated, sexually mature, male miniature swine severe hemorrhage model for potential improvement in rate and time of survival.
Methods: Fourteen swine were anesthetized, catheterized for hemorrhage and resuscitation and allowed to recover from anesthesia. The animals were sedated and hemorrhaged 60% of estimated blood volume (~39 ml kg^{-1}) exponentially over 1 h. Following hemorrhage the animals were treated with either 1.33 ml kg^{-1} of P188 (150 mg ml^{-1}; 7 swine) or the P188 citrate vehicle (7 swine) given as an i.v. infusion over 2 min without additional resuscitation fluids. The data were compared with control data from sedated swine similarly hemorrhaged, but with no resuscitation (untreated; $n = 16$).
Results: Median (95% CI) survival time for the untreated control swine was 55.8 (36.5–86) min with a 6% survival at 180 min. Median survival time of 161 (80–180) min for the P188 swine was significantly greater than control ($p = 0.0186$), whereas the citrate vehicle median survival time of 91 (32–180) min was not significantly different from control or P188. At the survival target time of 180 min, survival rates were not significantly different among the three groups. TEG data from swine demonstrated anti-coagulant properties of P188. This was confirmed with human blood *ex vivo*.
Conclusion: In the presence of severe controlled hemorrhage, P188 improved median survival time. However, retardation of blood clotting raises concerns as to its use in the presence of uncontrolled hemorrhage.

1. Introduction

Critical trauma resulting in severe hemorrhage on the battlefield or in remote domestic locations may require extended time for transport to adequate treatment facilities. Moreover, on-site emergency personnel (medics) may not have sufficient fluid volume for adequate hemorrhage resuscitation. Hemostasis, followed by hypotensive resuscitation [1,2] and low volume resuscitation (4–7 ml kg^{-1}) [3–5] are two techniques that require less resuscitation fluid and have promising outcomes, at least in the short term. In this study poloxamer 188 (P188) was evaluated as a small volume (1.33 ml kg^{-1}) bolus resuscitation product following severe hemorrhage in swine without additional resuscitation. A sedated, sexually mature male miniature swine severe hemorrhage model has been previously developed specifically for evaluating low volume resuscitation products, as part of the Defense Advanced Research Projects Agency (DARPA) Surviving Blood Loss program [6].

P188 is a non-ionic surfactant block copolymer of polyoxyethylene and polyoxypropylene that has rheologic, cytoprotective, anti-thrombotic and anti-inflammatory properties. Purified P188 has been used clinically in a number of medical applications including sickle cell disease [7]; cardiac membrane protection during surgery [8] and cardiac and skeletal muscle membrane repair in Duchenne muscular dystrophy [9,10]. It was shown in experimental animals to reduce cardiac infarct size [11]; improve neurologic outcome following hypothermic circulatory arrest [12]; improve survival following hemorrhage and resuscitation [13–16]. P188 is well tolerated by the body and is excreted almost exclusively by the kidney [17].

☆ A Spanish translated version of the summary of this article appears as Appendix in the final online version at doi:10.1016/j.resuscitation.2011.06.007.

☆☆ *Previous presentation*: An abstract and subsequent poster titled "Severe controlled hemorrhage resuscitation with small volume poloxamer 188 in sedated miniature swine," which included some of the data from this manuscript, was presented at the Shock Society annual meeting in Portland, OR in June 2010.

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0300-9572/$ – see front matter. Published by Elsevier Ireland Ltd.
doi:10.1016/j.resuscitation.2011.06.007

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01 NOV 2011	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Severe controlled hemorrhage resuscitation with small volume poloxamer 188 in sedated miniature swine	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
Burns J. W., Baer L. A., Jones J. A., Dubick M. A., Wade C. E.,	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8. PERFORMING ORGANIZATION REPORT NUMBER
United States Army Institute of Surgical Research, JBSA Fort Sam Houston, TX	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a REPORT	b ABSTRACT	c THIS PAGE			
unclassified	**unclassified**	**unclassified**	**UU**	**7**	

In the current study, swine were hemorrhaged 60% of estimated blood volume (EBV) exponentially over 1 h and then given P188 or its citrate vehicle. A target survival time of 180 min was specified by the DARPA program since it is anticipated that during military operations, recovery and transport of injured Soldiers or patients to trauma treatment areas and the first OR table could take at least 180 min. Based on previous studies, we hypothesized that small volume administration of P188 following severe hemorrhage would prolong survival time.

2. Materials and methods

This investigation and the previous model development protocol [6] were approved by the Institutional Animal Care and Use Committee of the U.S. Army Institute of Surgical Research, Fort Sam Houston, TX. All animals received care in strict compliance with the 1996 *Guide for the Care and Use of Laboratory Animals* by the National Research Council and were maintained in an Association for Assessment and Accreditation of Laboratory Animal Care International accredited facility. Also, blood was drawn from five healthy human volunteers for thromboelastogram (TEG) validation of swine TEG data with human blood, was in accordance with U.S. Army Institute of Surgical Research Command Policy 07-0008 (15 August 2007) using a Brooke Army Medical Center Institutional Review Board approved protocol.

Fourteen healthy, sexually mature, male Sinclair miniature swine with a mean weight of 41.0 ± 0.6 kg (±SEM) were obtained from Sinclair Research Center, Inc., Columbia, MO. Health of the animals was determined with a physical exam by a veterinarian, a lung CT scan and a blood sample for basic laboratory parameters (CBC/blood chemistry). The animals were acclimated to human activity, transport cages, laboratory procedures and being in a sling. The fourteen swine were given either P188 (7 swine) or citrate vehicle (7 swine) by i.v. infusion 2–3 min following hemorrhage with no additional resuscitation.

2.1. Experimental preparation

The experimental preparation has been described in detail previously [6]. Briefly: following 0.05 mg kg^{-1} of buprenorphine, 4–5 mg kg^{-1} of telazol, and isoflurane anesthesia, the animals were catheterized in a small branch of the right carotid artery with a Data Sciences International (DSI, St. Paul, MN) telemetry transducer for arterial blood pressure; the right external jugular vein for the continuous infusion of midazolam; the right femoral artery for hemorrhage and blood samples and the right femoral vein for blood samples and resuscitation. The catheters were tunneled subcutaneously to the dorsum over the shoulders and exteriorized. The incisions were closed with staples and infiltrated with bupivacaine. Isoflurane was discontinued and the animals were placed in a sling with feet on the floor and allowed to recover from anesthesia. Limb ECG electrodes were attached and BIS electrodes (Bispectral Index; Aspect Medical Systems, Newton, MA) were placed across the forehead. Midazolam infusion was started at 1.25 mg kg^{-1} h^{-1} and adjusted throughout the study to maintain a BIS sedation level of 80–90 [18]. After 30 min of stabilization, baseline hemodynamic data (systolic, diastolic and mean blood pressure and heart rate) were collected and baseline arterial and venous blood samples were taken for the following parameters: pO_2, sO_2, pCO_2, HCO_3, base excess (BE), pH, Hct, Hb, glucose, lactate, differential WBC and platelets, using standard CBC, blood gas co-oximetry and clinical chemistry techniques. The combined volume of arterial and venous blood taken for analysis was 26 ml per sample. The animals were then hemorrhaged 60% of their estimated blood volume (65 ml kg^{-1}) exponentially over 1 h [6,19] using a computer controlled withdrawal system.

2.2. P188 (n = 7) or citrate vehicle (n = 7) resuscitation

P188 was obtained as Flocor (SynthRx Inc., Bellaire, TX; 150 mg ml^{-1}) in an integral citrate buffer vehicle of 3.08 mg ml^{-1} sodium chloride, 2.38 mg ml^{-1} sodium citrate and 0.366 mg ml^{-1} citric acid (pH = 5.505; mOsm l^{-1} = ∼467). The P188 that we used was previously shown to prolong survival and reduce tissue injury in rats [16]. We also evaluated the citrate vehicle separately (pH = 5.631; mOsm l^{-1} = 126).

Immediately following hemorrhage, arterial and venous blood samples were collected over 2–3 min and either P188 (200 mg kg^{-1}; from Ref. [16]) or citrate vehicle was infused at 1.33 ml kg^{-1} over 2 min (3.4% of shed blood volume) with no additional resuscitation. The animals were observed and blood sampled at 15, 30, 60, 90, 120, 150, 180, 240, 300 and 360 min or until the animal expired. Hemodynamic data were sampled continuously. Death was defined as respiratory arrest. Any animal that survived beyond 360 min was euthanized with 10 ml of Fatal Plus (Vortech Pharmaceuticals, Dearborn, MI), i.v.

2.3. Thromboelastogram (TEG)

To evaluate whether P188 had an effect on coagulation, additional arterial blood samples (5 ml/time point) were taken from the pigs for TEG analysis at baseline before hemorrhage, immediately following hemorrhage and at 1 h following hemorrhage. The swine TEGs were abnormal following P188 administration. Therefore, to confirm the swine TEG values, 25 ml of blood was taken from each of 5 healthy human volunteers (3 males, 2 females) and treated with normal saline, the citrate buffer vehicle or P188 at the calculated dilution dose used in the swine and at one-half the calculated dose.

The whole blood coagulation assays were run at 37 °C using the TEG Hemostasis Analyzer 5000 (Hemoscope, Niles, IL). Swine blood was collected from the femoral artery silastic catheter and transferred into 3.2% sodium citrate Vacutainer tubes. The human blood was collected via venipuncture through a 19 g butterfly needle into 3.2% citrated syringes following a 3 ml waste. Swine and human blood samples were equilibrated at room temperature for 30 min to 1 h prior to analysis. Clotting was initiated by adding 20 μl of 0.2 M $CaCl_2$ and 10 μl of human recombinant tissue factor (Innovin, Dade Behring, Marburg, Germany diluted 1:200 with saline for swine or 1:500 with saline for human) to 340 μl of the sodium citrated blood samples. Triplicate samples were run at each time point and allowed to continue until at least 30 min after maximum clot strength was achieved. TEG parameters measured included: clot reaction time (R, time of initial fibrin formation detection); angle (α, rate of clot formation or clot kinetics); maximum amplitude (MA, maximum clot strength); and time to maximum amplitude (TMA, time needed to form a stable clot).

P188 and vehicle data were compared to each other and to a previously obtained untreated severe hemorrhage (60%) control model (n = 16) [6]. All 30 swine used in these comparisons (control, P188 and vehicle) were treated identically from baseline through the 60 min of hemorrhage.

2.4. Statistical analysis

Data are presented as mean ± SEM or median (95% CI), as appropriate. A within groups repeated measures analysis of variance was used to examine the difference among the control group and the P188 and vehicle resuscitation groups, including change over time and the interaction of time and group. The hemorrhage and the resuscitation/recovery periods were considered separately in the analysis. If the repeated measure ANOVA showed a significant difference between groups over time, a post hoc analysis

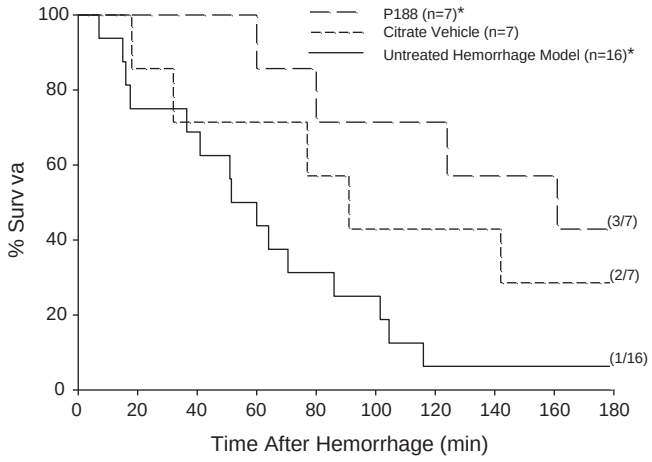

Fig. 1. Kaplan–Meier survival plot of control vs. P188 vs. citrate vehicle data. *Same symbol significant ($p < 0.05$) difference. At the target time of 180 min there were no significant differences among the three groups. Numbers in ( ) = number of survivors at 180 min for each group.

was performed to determine which time points were significantly different using an independent *t*-test between the groups (or non-parametric equivalent, as necessary). A Kaplan–Meier estimate of survival was constructed to compare the control vs. the P188 vs. the vehicle groups via the stratified log rank test. A $p \leq 0.05$ was considered statistically significant.

3. Results

3.1. Hemorrhage volume and survival

The 60% hemorrhage volume (ml kg^{-1}) of control, P188 and citrate vehicle swine was 38.8 ± 0.1, 39.1 ± 0.08 and 39.0 ± 0.2, respectively. The larger ml kg^{-1} volume for the P188 and citrate vehicle swine was the result of additional blood taken for TEG. Median (95% CI) survival time for the 16 control swine (no resuscitation) was 55.8 (36.5–86) min with a 6% (1/16) survival rate at the target time of 180 min. Median survival time of 161 (80–180) min for P188-treated swine was significantly greater than control ($p = 0.0186$; Fig. 1). However, the P188 survival rate of 43% (3/7) at 180 min was not significantly different than the control survival of 6% at 180 min ($p = 0.067$). The median survival time of 91 (32–180) min or survival rate (2/7; 29%) of animals administered vehicle was not different from control or P188.

3.2. Hemodynamics

Systolic blood pressure (SBP) from the P188 swine decreased from a baseline of 139 ± 8 mm Hg to 45 ± 6 mm Hg at the end of hemorrhage (similar to the data from the untreated control animals), and then significantly ($p < 0.05$) increased over control at 15 min and 30 min following P188 infusion, and remained higher than control through 90 min post hemorrhage (Fig. 2). Citrate vehicle SBP response was similar to P188 but was not significantly different from control or P188. MAP and diastolic pressure followed the same pattern. Heart rate was not different among the three groups during the entire study.

3.3. Respiratory and metabolic data

Hemorrhage resulted in a characteristic metabolic acidosis in all animals. In the P188 group, arterial pCO_2, HCO_3 and base excess (BE) decreased during hemorrhage (51 ± 2 to 31 ± 3 mm Hg; 33 ± 1 to 22 ± 2 mmol l^{-1}; and 7.9 ± 0.7 to -1.0 ± 0.9 mmol l^{-1}, respectively) but recovered significantly following P188 infusion compared to control (Fig. 2). Vehicle infused swine responded similarly to P188 with a recovery following infusion, but improvements in BE and HCO_3 were not sustained at 150 min. Although there were significant improvements in arterial pCO_2, HCO_3 and BE following P188 and vehicle infusion neither arterial nor venous pH showed any significant improvement following P188 or vehicle infusion and were not different than control.

There were no significant differences in arterial pO_2, sO_2, lactate, respiratory rate, Hct or Hb among control, P188 or vehicle animals during hemorrhage or recovery (Table 1). Although P188 and vehicle arterial glucose concentrations were significantly lower than control at baseline the magnitude of the change was similar through 30 min of recovery.

3.4. Coagulation

Control, P188 and vehicle values for PT (prothrombin time), aPTT (activated partial thromboplastin time), platelets and fibrinogen are presented in Table 2. Vehicle PT was significantly shorter than control and/or P188 throughout the study. P188 aPTT was significantly shorter than control and vehicle at baseline, remained shorter at end of hemorrhage, and then trended longer than control and vehicle throughout recovery, while vehicle platelet counts were significantly higher than control throughout the studies. Fibrinogen was not different among the three groups at any time (Table 2).

3.5. TEG data

Swine TEG values for the time to initiation of clot (R), the maximum rate of clot development (α) and the maximum amplitude of clot strength (MA) were similar at baseline and end of hemorrhage for P188 and the vehicle, but the P188 data changed dramatically at 1 h post-P188 infusion, whereas the vehicle data remained relatively unchanged (Table 3). Table 4 illustrates the R, α, MA and time to MA (TMA) from the TEG of human blood treated with saline, vehicle and P188 at the calculated swine dilution factor (1.0) and at one-half the dilution factor (0.5). Saline and the vehicle had no effect on the TEG data. However, P188 at 0.5 had a dramatic effect on the clotting mechanism, and clotting was non-existent with P188 at 1.0, even after an average of 141 min. The human blood TEG data supported the swine data indicating that the anti-coagulant activity observed is related to the P188 and not the vehicle.

4. Discussion

A severe controlled hemorrhage model with no resuscitation has been previously developed [6] specifically to evaluate small volume resuscitation products such as P188. In this study, P188, given as a small volume resuscitation product, significantly improved median survival time after severe controlled hemorrhage compared to control ($p = 0.0186$; Fig. 1). However, a P188 survival rate of 43% (3/7) at the target time of 180 min was not significantly different than the vehicle (29% or 2/7) or the control survival of 6% (1/16). These data suggest that since the P188 product used in this study contained both P188 and the citrate buffer vehicle, both components contributed to the longer survival times observed in the P188 group, but the mechanisms involved remain to be elucidated. However, citrate is well known for its anticoagulation activity and previous studies have indicated that anticoagulants, such as heparin, can improve survival from hemorrhage by improving microcirculatory flow [20]. Although we observed a significant increase in P188 median survival time compared to control, the primary end point of the study was not powered to detect significant differences in survival rate at the 180 target time. A power analysis based on the

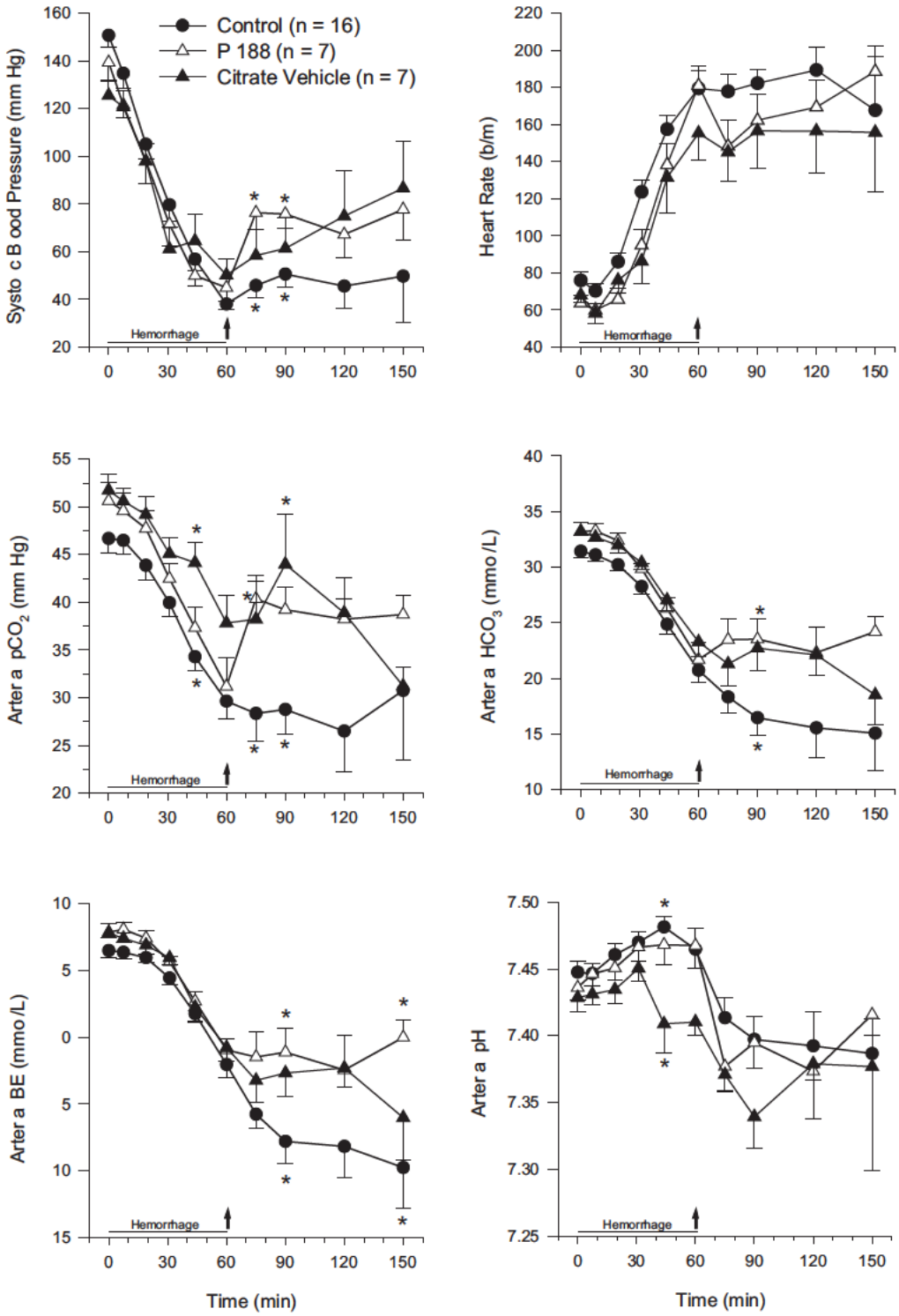

Fig. 2. Hemodynamic and metabolic data. Data are mean ± SEM. Arrow indicates time of 2 min infusion of P188 or citrate vehicle. *Same symbol significant ($p < 0.05$) difference.

survival rates of the current study indicated that an n of 31 swine in each group would be required for significance between the 6% control survival and the 43% P188 survival at 180 min. It should be noted that the three P188 animals that survived to 180 min also survived to 240 min, and one survived to 360 min. As mentioned, the 180 min survival target time threshold of the original DARPA surviving blood loss program was driven by the anticipated evacuation times for removal of severely injured personnel from austere forward conflict areas or from remote domestic locations.

P188 has been shown to decrease both the areas of coagulation and necrosis around a burn in experimental animals [21]; seal and repair membranes and block stress related cardiac failure in dystrophic mice [8–10,22]; improve neurologic outcome following hypothermic circulatory arrest [12]; reduce the size of myocardial infarct following coronary occlusion [11]; improve the vaso-occlusive crisis in sickle cell disease [7]; increase survival after hemorrhage and shock in anesthetized dogs [13,14], anesthetized rabbits [15] and unanesthetized rats [16].

Table 1
Respiratory and arterial metabolic data.

	Baseline	EOH	Post-hemorrhage recovery			
			15 min	30 min	60 min	90 min
pO_2 (mm Hg)						
Control	85.4 ± 1.8	97.8 ± 2.7	99.2 ± 4.0	100.4 ± 4.2	98.8 ± 5.6	93.4 ± 6.9
P188	80.5 ± 2.0	91.6 ± 5.0	96.5 ± 4.7	95.2 ± 4.3	94.2 ± 3.9	91.8 ± 4.0
Vehicle	84.9 ± 2.0	95.7 ± 7.9	94.8 ± 7.0	82.0 ± 9.6	88.6 ± 6.9	101.5 ± 5.6
sO_2 (%)						
Control	96.9 ± 0.3	97.9 ± 0.2	97.6 ± 0.4	97.7 ± 0.3	97.5 ± 0.3	97.4 ± 0.6
P188	96.3 ± 0.3	97.0 ± 0.6	97.0 ± 0.5	97.0 ± 0.3	96.8 ± 0.2	96.9 ± 0.2
Vehicle	96.4 ± 0.6	96.1 ± 1.2	95.6 ± 1.5	94.8 ± 2.0	94.8 ± 1.6	96.1 ± 1.5
Resp. Rate (breaths min^{-1})						
Control	26 ± 1.2	22 ± 2.0	24 ± 3.1	27 ± 3.9	33 ± 4.0	29 ± 5.3
P188	22 ± 2.8	21 ± 3.5	21 ± 4.0	28 ± 3.9	26 ± 5.0	22 ± 3.8
Vehicle	25 ± 2.4	18 ± 2.3	17 ± 2.3	21 ± 4.3	22 ± 4.5	20 ± 6.1
Lactate (mmol l^{-1})						
Control	1.0 ± 0.1	8.3 ± 0.9	11.4 ± 1.1	12.1 ± 1.3	12.5 ± 2.2	14.9 ± 3.4
P188	1.2 ± 0.1	7.2 ± 0.6	9.7 ± 1.6	9.7 ± 1.7	11.5 ± 2.3	8.8 ± 1.8
Vehicle	1.0 ± 0.1	7.9 ± 1.1	9.7 ± 1.7	9.2 ± 1.8	9.5 ± 2.1	10.4 ± 2.6
Glucose (mmol l^{-1})						
Control	5.1 ± 0.1*,#	11.9 ± 1.0*	11.5 ± 1.0*	10.8 ± 1.1*	7.3 ± 1.1	7.2 ± 0.7
P188	4.2 ± 0.3*	7.9 ± 1.2*	6.7 ± 1.0*	5.8 ± 0.7*	6.0 ± 1.1	7.1 ± 1.4
Vehicle	4.2 ± 0.2#	9.6 ± 1.3	8.1 ± 1.2	7.8 ± 1.3	9.2 ± 1.2	8.8 ± 0.8
Hct (%)						
Control	41.6 ± 1.2	41.8 ± 1.4	41.4 ± 1.6	39.8 ± 1.5	40.4 ± 1.8	39.6 ± 1.4
P188	37.4 ± 1.3	44.3 ± 2.7	35.3 ± 1.6	34.7 ± 1.1	37.4 ± 1.0	38.6 ± 1.3
Vehicle	38.1 ± 0.8	41.6 ± 1.2	41.7 ± 1.1	42.7 ± 0.3	43.0 ± 0.8	43.3 ± 0.9
Hb (g dl^{-1})						
Control	12.1 ± 0.3	12.1 ± 0.5	11.8 ± 0.5	11.5 ± 0.5	11.7 ± 0.6	11.2 ± 0.5
P188	11.0 ± 0.3	12.7 ± 0.6	10.2 ± 0.5	10.0 ± 0.4	10.7 ± 0.3	11.1 ± 0.4
Vehicle	11.3 ± 0.3	12.0 ± 0.3	11.6 ± 0.2	12.1 ± 0.1	12.0 ± 0.3	12.3 ± 0.3

Data are mean ± SEM; control ($n = 16$); P188 ($n = 7$); citrate vehicle ($n = 7$); EOH = end of hemorrhage; *,# = same symbol significant difference ($p \leq 0.05$).

Table 2
Coagulation data.

	Baseline	EOH	Post-hemorrhage recovery			
			15 min	30 min	60 min	90 min
PT (s)						
Control	10.9 ± 0.2*,#	11.3 ± 0.2*	11.5 ± 0.2*	11.5 ± 0.2*	11.6 ± 0.3*	11.7 ± 0.5*
P188	10.8 ± 0.1#	11.1 ± 0.1	11.8 ± 0.2#	11.9 ± 0.2#	11.7 ± 0.2#	11.4 ± 0.2
Vehicle	8.9 ± 0.7*,#	9.5 ± 1.0*	9.4 ± 0.7*,#	9.1 ± 0.9*,#	8.3 ± 0.9*,#	8.5 ± 0.9*
aPTT (s)						
Control	15.9 ± 0.1*	16.4 ± 0.4	18.3 ± 1.1	18.1 ± 1.0	17.3 ± 1.5	15.5 ± 0.5
P188	13.9 ± 0.2*,#	14.4 ± 0.2	21.9 ± 2.0	21.3 ± 3.1	19.4 ± 1.9	16.3 ± 0.6
Vehicle	15.6 ± 0.6#	16.5 ± 0.9	18.4 ± 1.4	18.0 ± 1.2	16.7 ± 1.3	15.9 ± 0.7
Platelets (10^3 μl^{-1})						
Control	383 ± 14.4*	248 ± 12.4*,#	247 ± 15.0*	266 ± 10.9*	259 ± 14.1*	248 ± 17.7*
P188	458 ± 29.6	362 ± 23.6*	295 ± 19.5	306 ± 22.3#	320 ± 24.0	339 ± 22.2
Vehicle	493 ± 41.6*	390 ± 19.2#	368 ± 26.6*	378 ± 25.0*,#	376 ± 32.5*	388 ± 43.5*
Fibrinogen (mg dl^{-1})						
Control	348 ± 36.4	279 ± 25.7	269 ± 24.6	287 ± 28.5	240 ± 3.6	256 ± 30.1
P188	343 ± 8.9	259 ± 18.2	273 ± 24.5	251 ± 12.0	246 ± 16.7	263 ± 19.8
Vehicle	300 ± 20.7	243 ± 27.6	258 ± 30.5	241 ± 14.5	245 ± 18.2	247 ± 20.6

Data are mean ± SEM; control ($n = 16$); P188 ($n = 7$); citrate vehicle ($n = 7$); EOH = end of hemorrhage; PT = prothrombin time; aPTT = activated partial thromboplastin time; *,# = same symbol significant difference ($p \leq 0.05$).

Each of the previous hemorrhage studies in experimental animals, although differing in protocol, showed survival improvement with the use of P188 [13–16]. The study most similar to the current small volume P188 study utilized anesthetized mongrel dogs given an injection of 1 ml kg^{-1} of a 5% pluronic F-68 (P188) saline solution following hemorrhage to 50 mm Hg [13]. The only significant observation over the 2 h recovery period in that study was an increase in renal blood flow, considered to be a rheological effect of the pluronic F-68. In contrast to the present small volume P188 resuscitation study where P188 was used alone without additional resuscitation, the anesthetized beagles [14] and rabbits [15] and the unanesthetized rats [16] were resuscitated with P188 in combination with blood, normal saline, LR or Hextend.

Table 3
TEG data.

	Baseline	EOH	1 h post
R (min)			
P188	8.4 ± 1.0	9.1 ± 2.9	33.7 ± 13.2
Vehicle	5.6 ± 0.8	4.7 ± 0.5	7.2 ± 1.0
α (degree)			
P188	53.3 ± 5.1	53.1 ± 9.5	10.0 ± 4.0*
Vehicle	66.7 ± 3.3	68.8 ± 1.8	59.9 ± 2.4
MA (mm)			
P188	75.3 ± 0.9	63.7 ± 3.4	7.0 ± 1.9*
Vehicle	74.7 ± 1.5	69.7 ± 1.7	66.1 ± 2.8

Data are mean ± SEM; P188 ($n = 7$); citrate vehicle ($n = 7$); EOH = end of hemorrhage; *R* = time to initiation of clot; α = maximum rate of clot development; MA = maximum amplitude of clot development.
* Significantly different than vehicle.

Table 4
Human blood TEG data.

	Saline (0.5)	Saline (1.0)	Vehicle (0.5)	Vehicle (1.0)	P188 (0.5)	P188 (1.0)
R (min)	7.8 ± 0.5	7.4 ± 0.3	7.7 ± 0.4	7.8 ± 0.6	10.9 ± 0.9	141.2 ± 67.3
α (degree)	57.0 ± 1.7	58.2 ± 1.5	53.4 ± 1.4	55.4 ± 2.3	15.5 ± 5.7	–
MA (mm)	60.1 ± 2.1	60.9 ± 1.9	58.6 ± 2.0	59.8 ± 1.9	6.7 ± 2.9	–
TMA (min)	27.2 ± 1.4	27.2 ± 0.8	28.4 ± 0.4	28.8 ± 1.1	13.6 ± 0.4	–

Data are mean ± SEM; human blood ($n = 5$) treated with saline, citrate vehicle or P188 at the calculated dilution dose used in the swine (1.0; 6 mg ml^{-1}) and at one-half the calculated dose (0.5; 3 mg ml^{-1}); *R*, α and MA are the same as Table 3; TMA = time to maximum clot amplitude. The P188 (1.0) column indicates that there was no clotting, even after an average of 141 min.

Severe hemorrhage often leads to acidosis and coagulopathy [23,24], microvascular hypoperfusion and ischemia, with potential end-organ damage. The protective mechanism of P188 following hemorrhagic shock appears to be multifaceted through its surfactant, cytoprotective, rheologic, anti-inflammatory and antithrombotic properties [25,26]. The surfactant properties of P188 have been reported to reduce RBC adhesions, platelet aggregation [27] and blood surface tension [14], increasing microcirculation. In addition, P-188 may have drag-reducing properties. Several studies have indicated that drag-reducing polymers can increase blood flow, tissue perfusion in ischemia/reperfusion models [28] and improve survival after hemorrhagic shock in animal models [29].

The TEG data in our study indicate that the P188 formulation used had a negative effect on coagulation, possibly by interference with the Hageman factor (coagulation factor XII) [27], and prolongation of aPTT [30]. Although citrate has anti-coagulant properties, the plasma concentration of the citrate buffer vehicle in this study was apparently not great enough to have a systemic anticoagulation effect. Thus, the anti-coagulation effects illustrated in Tables 3 and 4 are related to the current P188 formulation used, and not specifically to the citrate buffer alone. Interestingly, rats pretreated with 75 mg kg^{-1} of P188 followed by 150 mg kg^{-1} h^{-1} of P188 for 60 min after 75% tail amputation showed no increase in spontaneous bleeding [16]. TEG data were not collected. Possibly, with the additional resuscitation used, the P188 plasma concentration was not high enough for anti-coagulation activity, or the rat response to P188 may differ from swine and humans. Additional experiments would need to be performed in uncontrolled hemorrhage models to determine the physiological significance of the anti-coagulation properties observed by TEG.

4.1. Limitations

The animals in this study were sedated rather than anesthetized. Consequently, we could not perform extensive invasive procedures to investigate the hemodynamic, metabolic or other potentially beneficial effects of P188 or its citrate buffer vehicle. Also, the absence of concurrent controls is recognized as not being optimal. However, the control data were collected over a 21 month period and were highly consistent as to survival time, with a 6% survival rate at the designated time of 180 min. In light of these data and the need to reduce animal use, we have opted to employ historical untreated controls in the present study. Moreover, all animals used in these comparisons were treated identically from baseline through end of hemorrhage. This study was designed to screen potential agents used in small volumes that can improve survival time, with the intent to buy time to get the patient to a definitive treatment facility. For the DARPA program, this time was 180 min. The survival rates obtained indicated that much larger group sizes would be required to power the study sufficiently to show a significant difference in survival rates, which was beyond the scope of our study.

4.2. Summary

The previously developed sedated, sexually mature male miniature swine model, designed as a screening tool to evaluate small volume resuscitation following severe controlled hemorrhage, was successfully employed in this study. The results support previous P188 studies with an increase in severe hemorrhage median survival time using small volume P188 resuscitation, although the majority of previous hemorrhage studies resuscitated with P188 used additional fluids (shed blood, lactated Ringer's, Hextend, saline or larger volumes or higher concentrations) to maintain a predetermined blood pressure for a specific time. Our results also suggest that the citrate vehicle, a component of the P188 formulation used, contributed to the improved survival time seen in the P188 group.

Medical support personnel responding to trauma on the battlefield or in remote domestic locations may not have adequate fluids for resuscitation, or may only have a short time to stay with the wounded (battlefield) and would benefit from a small volume resuscitation product that could be given after hemorrhage control to sustain the wounded for extended time before evacuation to a medical support facility. However, P188 may have limited potential, especially in the presence of uncontrolled hemorrhage, due to its negative effect on coagulation. This aspect of its actions would need additional study if P188 is to be developed further as an adjunct to fluid resuscitation strategies.

Conflict of interest statement

None of the authors have a conflict of interest in this study or this manuscript.

Funding

Defense Advanced Research Projects Agency (DARPA) Surviving Blood Loss (SBL) Program.

Acknowledgements

We wish to thank personnel of the Veterinary Support Division and the Laboratory Support Division, U.S. Army Institute of Surgical Research for their support in the maintenance, training, animal monitoring (Belinda Meyers), surgery (Johnny Nelson, Jr.) and blood chemistry analyses during these investigations. Also, we thank Chriselda Fedyk and Michael Scherer for the TEG analyses.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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